Hair Element Concentrations and Young Children's Classroom and Home Behavior Mike Marlowe, Ph.D.¹ and Leonard B. Bliss, Ph.D.²

Abstract

There is a growing interest in the use of hair element analysis as a medical and behavioral diagnostic tool. Recent studies have found significantly abnormal levels of trace minerals and toxic metals in the head hair of children with learning and behavior disorders. Utilizing hair element analysis, this study examined six toxic metals and 14 trace minerals in relation to teacher and parent ratings of preschool children on the Walker Problem Behavior Identification Checklist. Parents were interviewed to control for confounding variables that might mask the relationship between hair element status and behavioral development. Regression analysis indicated that the set of hair elements was significantly related to increased scores on the teacher and parent rated behavior scales with increased hair lead concentrations being the major contributor to the dependent measures.

In recent years there has been a growing interest in the use of hair element analysis as a medical and behavioral diagnostic tool. Recent studies have found significantly abnormal levels of trace minerals and toxic metals in the head hair of children diagnosed with learning and behavioral disorders (Kracke, 1982; Marlowe, Errera, Cossairt, and Welch, 1984; Marlowe, Schneider, and Bliss, 1991; Shauss, 1981a; Thatcher and Lester, 1985). Trace minerals influence and may even regulate enzyme activity which, in turn, may influence behavior through biochemical processes such as neurotransmitter activity and neuronal transmission. Toxic metals such as lead, cadmium, and aluminum are commonly implicated in behavioral disorders and are known to disrupt neurochemical and biochemical functioning.

The primary purpose of the present study was to further examine the relationship between

hair elements and behavioral performance in children. Concentrations of hair elements were examined in relation to teacher and parent ratings of preschool children on the Walker Problem Behavior Identification Checklist.

A second purpose was to extend the datum on hair element reference values for young children. Reported hair element reference values have, in the main, been based on healthy adults, and there is a need for better reference values for young children.

Method

Subjects

The 102 subjects were randomly drawn from eight preschools in the northwestern region of North Carolina. Parental consent was required for study participation. Interviews with their teachers indicated none of the children were receiving special education or related services for behavioral disorders.

Control of Confounding Variables

Parents of subjects were interviewed via questionnaire in order to identify and control for the following confounding variables affecting behavioral development: history of immunizations against childhood diseases, length of hospitalization at birth, birth weight, number of hospital admissions since birth, history of pica (ingestion of non-nutrative substances), birth order, number of siblings, presence of father in the home, and mother and father's occupation and level of educational attainment. Table 1 contains the relevant demographic data for the 102 subjects.

Classification of Element Levels

After obtaining parental permission, children were asked to submit a small sample of hair (about 400 mg) for trace element analysis. Hair samples were collected from the nape of the child's neck, as close to the scalp as possible, by the senior researcher, using stainless-steel scissors. The hair samples were

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Percent male

Percent Caucasian

Percent completed immunization history	100.0	
Percent father present in home	78.6	
Percent positive pica history	7.8	
	Mean	SD
Age (years)	3.48	0.67
Birthweight (ounces)	119.11	14.50
Length of hospitalization	1.92	0.97
Birth order	1.66	0.61
Number of hospital admissions	0.17	0.40
Number of siblings	1.02	0.77
Father's education level (grades)	14.94	2.37
Mother's education level (grades)	14.87	2.11
Father's social class (2-factor Hollingshead)	2.41	0.80
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Table 1Subjects' Demographic Characteristics

then submitted to Omega Tech, a state and Center for Disease Control licensed laboratory in Trout Dale, Virginia, and a member of the Hair Analysis Standardization Board of the American Society of Elemental Testing Laboratories (ASETL). Hair samples were analyzed with the atomic absorption spectrophotometer, the graphite furnace, and the inductively coupled argon plasma quantimeter (ICP) to determine the following elements: lead, cadmium, mercury, aluminum, arsenic, nickel, copper, zinc, iron, chromium, manganese, cobalt. lithium, molybdenum, phosphorus, selenium, calcium, magnesium, sodium, potassium, and vanadium.

Hair element analysis is established as a screening test for contamination with lead, cadmium, and other toxic metals (Laker, 1982; U.S. Environmental Protection Agency, 1980), but its usefulness for defining nutritional status remains to be defined (Kelvay, Bistrian, Fleming, and Neuman, 1987). Scalp hair has several characteristics of an ideal tissue for epidemiologic study in that it is painlessly removed, normally discarded, easily collected, and its contents can be analyzed relatively easily. Trace elements are accumulated in hair at concentrations that are generally higher than those present in blood or urine (Passwater and Cranton, 1983). Elements once situated in the hair are no longer in dynamic equilibrium with the body since hair is a metabolic end product (Katz and Chart, 1988). Hair, thus, may provide a record of exposure over time to

toxic metals and a continuous record of nutrient mineral status since hair grows at the rate of approximately 1.0 cm per month (Myers and Hamilton, 1951).

52.4

98.1

100 0

The Walker Problem Behavior Identification Checklist

The Walker Problem Behavior Identification Checklist (WPBIC) (Walker, 1983) was used to obtain teacher and parental ratings of each child's behavior. Although developed for use with teachers, the WPBIC has also been used reliably with parents (Christopher-sen, Barnard, Ford, and Wolf, 1976; Strain, Steele, Ellis, and Timm, 1982). The checklist contains 50 statements about behavior (e.g., "must have last word in verbal exchanges,", "steals things from other children", and "comments that no-one understands him"). The person completing the checklist indicates when a statement describes the child's behavior in the last two months. Weighted scores based on the relative importance of each item are then summed for each of five scales: Acting Out, Withdrawal, Distractibility, Disturbed Peer Relations, and Immaturity. Scale scores can then be added together to give an overall measure of behavioral functioning. Normative data for the test is based on 1,882 children, aged 2-12 years 1983); and separate T-score (Walker, transformations are available for both boys and girls aged 2-6 years, 7-9 years, and 10-12 years for each scale score and the total behavioral score.

Reliability and validity data available for the test suggest that the scale is a valid and reliable predictor of behavior disturbance in children. A split-half reliability coefficient of .98 is reported (Walker, 1967), as is a test-retest reliability of .86 (Bolstad and Johnson, 1977). Total score stability coefficients approximate to .80 across separate studies and samples (Walker, 1983). Similarly a correlation of .81 has been found between teacher and parent ratings of child behavior (Strain et al., 1982). Content, criterion, construct, factorial, and item validity measures are also available. For example, Bolstad and Johnson (1977) found that children scoring high and low on the checklist differed significantly in classroom behavior, and similar results have been found by Walker (1983). Parent ratings on the checklist have also been found to discriminate

between behaviorally deviant and behaviorally acceptable boys (Mash and Mercer, 1979). Evidence thus suggests a strong relationship between the child's actual behavior at home and school and the ratings given on the checklist (Walker, 1983).

In this study, classroom teachers and parents were instructed by the senior researcher to fill out the scale. All ratings were based on observations of the child's school and home behavior for the past two months prior to hair collections.

Results

The 102 subjects' mean hair element levels are shown in Table 2 along with the accepted upper limits for hair metals and the theoretical normal ranges for hair minerals established by the laboratory (Omega Tech, 1992). No

			Omega Tech	Doctors Data	
	Mean	SD	Normal Range	Normal Range	
Element	(ppm)	(ppm)	(ppm) ^a	(ppm) ^c	
Calcium	119.265	76.571	200.00 - 600.00	125.00 - 300.00	
Magnesium	8.979	6.189	25.0-75.0	25.00 - 28.00	
Zinc	74.647	42.850	160.0 - 240.0	90.00-135.00	
Copper	25.192	21.932	12.0-35.0	8.00-15.00	
Chromium	0.178	0.075	0.5-1.5	0.78 - 1.00	
Sodium	94.039	148.074	150.0-350.0	18.00-35.00	
Potassium	84.860	121.761	75.0-180.0	12.00 - 40.00	
Selenium	0.121	0.054	3.0-6.0	0.39-0.70	
Manganese	0.350	0.267	1.0- 10.0	0.26 - 0.52	
Cobalt	0.105	0.045	0.2-1.0	0.26 - 0.47	
Iron	17.161	8.598	20.0 - 50.0	10.00 - 20.00	
Lithium	0.005	0.002	0.1 -0.8	0.02-0.13	
Molybdenum	0.100	0.003	0.1-1.0	0.21 -0.44	
Phosphorus	99.961	12.505	100.0 - 170.0	110.00-160.00	
Vanadium	0.052	0.037	0.5-1.0	0.36-0.80	
Lead	1.634	1.266	20.0 ^b	3.00 ^d	
Mercury	0.424	0.135	2.5 ^b	2.50^{d}	
Cadmium	0.263	0.219	1.0^{b}	1.00^{d}	
Arsenic	1.107	0.253	2.0^{b}	7.5^{0d}	
Nickel	0.337	0.283	1.0^{b}	1.00^{d}	
Aluminum	15.627	7.967	20.0 ^b	10.0^{d}	

Table 2Results of Hair Element Analysis

a. Theoretical normal range for adults (+1 SD) established by Omega Tech (1992).

b. Normal tolerated limit for adults established by Omega Tech (1992).

c. Theoretical normal range for children, ages one to five years, (+1 SD) established by Doctors Data, Inc. (1993).

d. Normally tolerated limit for children, ages one to five years, established by Doctors Data, Inc. (1993).

subjects evidenced hair lead, cadmium, arsenic, or mercury levels above the accepted upper limits, while 25 subjects were elevated in hair aluminum (> 20 ppm).

The subjects' mean hair concentrations of calcium, magnesium, zinc, chromium, sodium, selenium, manganese, cobalt, iron, lithium, phosphorous, and vanadium were more than one standard deviation below the theoretical normal ranges for these hair elements. Copper, potassium, selenium, and molybdenum mean hair concentrations were within the theoretical normal range (+ 1 SD).

It is important to note that Omega Tech's laboratory norms are based on a sample population of healthy adults. Norms for preschool children have not been established. Doctors Data, Inc., a state and CDC licensed laboratory in West Chicago and a member of the Hair Analysis Standardization Board of ASETL, has established laboratory norms for children ages one to five years (Doctors Data, 1993). These are also presented in Table 2. According to Doctors Data's preschool norms, 75 of the 102 subjects had elevated hair aluminum concentrations (> 10 ppm). Eight subjects evidenced elevated hair-lead concentrations (> 3 ppm); no subjects were elevated in cadmium, mercury, arsenic, or nickel. Regarding trace minerals, the subjects' mean hair concentrations of calcium, magnesium, zinc, chromium. selenium, cobalt. lithium. molybdenum, phosphorous, and vanadium were more than one standard deviation below the normal reference range. Copper, sodium, and potassium concentrations were elevated (> 1 SD above normal). Manganese and iron mean hair concentrations were within the theoretical normal range.

WPBIC scores are shown in Table 3. All mean scale scores and the total scale scores were within the range of normal behavior as rated by both teachers and parents.

While birth order and social class were uncorrelated, each was positively correlated with several of the WPBIC scales. All other confounding variables were uncorrelated with WPBIC ratings or hair element levels.

	Teacher		Parent		Raw score denoting disturbed behavior	
Scale	Mean	SD	Mean	SD	(+1 SD above mean)	
Acting out	1.55	2.02	1.05	1.95	10	
Withdrawal	0.58	1.30	0.58	1.12	4	
Distractibility	2.67	2.32	1.84	2.07	5	
Disturbed Peer Relations	1.01	1.66	0.85	1.43	3	
Immaturity	1.17	1.77	0.79	1.20	3	
Total Scale	7.00	6.98	5.11	5.23	20	

Table 3 Walker Problem Behavior Identification Checklist Scores

Table 4Pearson's Correlation

Mineral	Teacher Total Scale Score	Parent Total Scale Score	
Calcium	.34**	.19*	
Magnesium	.23*	.19*	
Copper	.35**	.34**	
Manganese	.25*	.23*	
Iron	.29**	.25*	
Lead	.73**	.65**	
Cadmium	.35**	.25*	
* p < .05			
**p < .01			

Pearson product correlations were also computed between the WPBIC total scale scores and the set of hair elements. These correlations are shown in Table 4. The following hair elements, listed in order of magnitude, correlated significantly and positively with both teacher and parent total scale scores: lead, copper, aluminum, cadmium, iron, calcium, manganese, and magnesium. Molybdenum correlated significantly and positively with teacher total scale score as did vanadium. All other hair elements were uncorrelated with the WPBIC measures.

A hierarchical multiple regression analysis using sets of predictor variables was performed on each of the five scale scores in addition to the total scale score. The incremental increase in explained variance of each criterion variable attributable to the set of hair elements over and above the control variables of birth order and social class was tested for significance. As a check on some of the assumptions underlying the use of multiple regression, scatterplots were constructed between each of the WPBIC Scale distributions and each of the predictor variables. No patterns of curvilinearity were detected.

Residual plots were generated to test the equality of variance assumption and to identify outliers. The variability of standardized residuals was uniform over all cases for each criterion scale distribution. An outlier was defined as a case falling at least 2 standard deviations from the mean of the residual distribution. Less than 3% of the cases were identified as outliers, and the cases were not constant across scales.

Although a moderately positive skew was

		df	\mathbf{F}	Р	\mathbf{R}^2	CumR ²
			Acting Out			
Lead		1,98	42.69	<.001	.30	.30
Iron		2,97	10.21	<01	.06	.36
Manganese		3,96	4.37	<05	.04	.40
Calcium		4,95	5.73	<05	.04	.44
			Withdrawal			
Lead		1,98	16.2	<.001	.20	.20
Cadmium		2,97	5.29	<05	.05	.25
			Distractibility			
Lead		1,98	50.12	<.001	.33	.33
Iron		2,97	4.60	<05	.03	.36
Copper		3,96	4.79	<05	.03	.39
Molybdenum		4,95	4.34	<05	.03	.42
-		Ľ	listurbed Peer Rela	ations		
Lead		1,98	60.47	<.001	.38	• .38
Molybdenum		2,97	9.74	<01	.04	.42
-			Immaturity			
Lead		1,98	57.79	<.001	.37	.37
Iron		2,97	33.17	<001	.03	.40
			Total Scale			
Lead		1,98	21.4	<001	.53	.53
Chromium		2,97	13.27	<.001	.03	.56
Molybdenum		3,96	5.75	<.05	.02	.58
Iron	4,95		5.59	<05	.02	.60

Table 5 Regression Results for Teacher WPBIC Responses

detected in some of the scale distributions, the F test is robust with respect to violations of normality, and correlations will not be deflated appreciably unless the skewness of one variable is opposite the skewness of the other variable. None of the predictor variables revealed a negative skew.

The results of the regressions on the teacher and parent ratings of the WPBIC are shown in Tables 5 and 6, respectively. The tables show the contribution of each mineral element to the variability of each scale over and above that of the control variables.

For the teacher rated acting out scale, lead accounted for 30% of the variance with iron accounting for an additional 6%, manganese 4%, and calcium 4%. For the parent rated acting out scale, lead accounted for 17% of the variance with chromium accounting for an additional 10%, molybdenum 4% and iron 4%.

For the teacher rated withdrawal scale, lead

accounted for 20% of the variance with cadmium accounting for an additional 5%. For the parent rated withdrawal scale, lead accounted for 12% of the variance with aluminum accounting for an additional 8%.

For the teacher rated distractibility scale, lead accounted for 33% of the variance with iron accounting for an additional 3%, copper 3%, and molybdenum 3%. For the parent rated distractibility scale, lead accounted for 29% of the variance, iron an additional 10%), and vanadium 3%.

For the teacher rated disturbed peer relations scale, 38% of the variance was attributable to lead with an additional 4% attributable to molybdenum. For the parent rated disturbed peer relations scale, 13% of the variance was attributable to lead and an additional 4% to potassium.

For the teacher rated immaturity scale, 3 7%> of the variance was attributable to lead with

Table 6 Regression Results for Teacher WPBIC Responses								
	df	F	Р	\mathbf{R}^2	CumR ²			
 		Acting Out	Į					
Lead	1,98	21.40	<001	.17	.17			
Chromium	2,97	13.27	<002	.10	.27			
Molybdenum	3,96	5.75	<.05	.04	.31			
Iron	4,95	5.59	<05	.04	.35			
_		Withdrawal			_			
Lead	1,98	14.31	<001	.12	.12			
Aluminum	2,97	8.77	<.01	.08	.20			
		Distractibility						
Lead	1,98	40.68	<.001	.29	.29			
Iron	2,97	15.39	<.001	.10	.39			
Vanadium	3,96	4.96	<.05	.03	.42			
Disturbed Peer Relations								
Lead	1,98	15.52	<.001	.13	.13			
Potassium	2,97	5.27	<05	.04	.17			
Immaturity								
Lead	1,98	31.14	<.001	.24	.24			
Nickel	2,97	4.13	<.05	.03	.27			
Potassium	3,96	4.75	<.05	.03	.30			
Total Scale								
Lead	1,98	39.95	<001	.41	.41			
Chromium	2,97	28.93	<001	.03	.44			

an additional 3% attributable to iron. For the parent rated immaturity scale, 24% of the variance was attributable to lead, 3% to nickel, and 3% attributable to potassium.

For the teacher rated total scale, 54% of the variance was attributable to lead with an additional 3% attributable to vanadium, 2% to molybdenum and 2% to aluminum. For the parent rated total scale, 40% of the variance was attributable to lead, and 3% to chromium.

Discussion

This study did not establish a causative relationship but showed an association between hair element concentrations, most notably hairlead, and nonadaptive classroom and home behavior in children. Regression analysis indicated that hair lead concentrations were significantly and positively related to increased teacher and parent rating scores on the five scales and total scale of the WPBIC.

The role of lead toxicity in behavioral development is well established. Needleman and others (1979) offered evidence that lead exerts its neurotoxic effects over a continuum. Part of their 1978 study examined the relationships between teachers' ratings of first and second grade children on an informal 11 -item classroom behavior scale and children's dentine lead levels (N = 2,146). The relationship of negative reports increased in a dose related fashion for all 11 items. A ten year follow up study of these children demonstrated that the effects of low level lead exposure persist (Needleman and Allred, 1990). When these subjects were reexamined it was found that subjects with dentine lead levels greater than 20 ppm in 1978 had markedly higher risk of dropping out of school, lower class standing, greater absenteeism, and impairment of fine motor skills as compared to those subjects who had dentine lead levels less than 10 ppm in 1978. Other general population studies have broadly replicated Neddleman et al.'s 1978 findings (Marlowe, Cossairt, Moon, et al., 1985; Silva, Hughes, Williams, and Faed, 1988; Yule, Lansdown, Millar, and Urbanowicz, 1981), and studies of behaviorally disordered populations have reported increased lead levels (Hansen, Christensen, and Tarp, 1980; Kracke, 1982; Marlowe, Schneider and Bliss, 1991; Schauss, 1981a). These observations replicated by

independent investigators in different cultures lend support to the causal inference.

It is important to reiterate that the mean hair lead level of the sample population was well within the laboratories' accepted upper limits of exposure for adults and young children. Recent neurochemical studies of low-level lead exposure have confirmed, however, that lead at low levels is a potent neurotoxin (Silbergeld and Hruska, 1980). The neurotoxic effects of lead can occur before histopatho-logical alterations are present.

The neurotoxic effects of lead are demonstrable in neuronal systems using acetylcholine, catcholaimines, and GABA as transmitters. These systems have been studied extensively and appear to offer some correlations between observed physiological and neurobehavioral impairments and neurochemistry. Neurochemical studies caution against assuming the existence of a "safe" level of lead exposure and raise concerns that the neuron may be irreversibly damaged by any exposure to lead.

The data on low level lead toxicity have been sufficiently convincing to deliberative and regulatory bodies in the United States and abroad that these agencies have in recent years promulgated increasingly stringent recommendations concerning reduction of lead exposure. In their review of this topic the United States Centers for Disease Control (1991) concluded that "as yet, no threshold has been identified for the harmful effects of lead" (p.2).

The behavioral disorders described in clinical and experimental lead poisoning are extremely variable and complex. This study also demonstrated such variability, inasmuch as WPBIC scales measuring such varied behaviors as acting-out and withdrawal were significantly related to increases in hair-lead. It may be that one should consider the nature of lead-induced changes as a randomization of behavioral responses or as a generalized hyperactivity. This hyperactivity would be situation-dependent and highly responsive to sensory stimuli, which might account for the variability reported in this and other behavioral studies.

The subjects had significantly reduced hairconcentrations of calcium, zinc, copper, and iron, and research indicates that a dietary deficiency or insufficiency of these nutrient minerals potentiates the toxicity of lead (Quillen, 1987). These nutrients moderate lead poisoning through mechanisms which determine rates of absorption and excretion and mechanisms which determine rate and tissue site of lead deposition. Although no dietary data was obtained, it may be that the nutrient deficiencies noted in the screening may have contributed to low lead levels negative relationship to behavioral performance.

Increased hair aluminum concentrations were a significant contributor to the teacher rated total scale score. It is remarkable that 24.5% of the subjects were elevated in hair-aluminum utilizing the Omega Tech adult laboratory norms, while 74.5% were considered elevated according to the preschool norms of Doctors Data. Doctors Data's normally tolerated limit for hair-aluminum in young children was established through hair element profiles of normal, healthy young children (N= 535) and through laboratory sponsored studies linking moderately increased hair aluminum concentrations to decrements in children's psychological performance. These studies reported hair-aluminum mean values of 9.53 (Marlowe, Stellern, Moon, and Errera, 1985), 9.33 (Marlowe, 1992), and 9.41 (Marlowe, Cossairt, et al., 1985). The sample population's high incidence of elevated hair-aluminum values is unexplained, but various exposures with aluminum sulphate in drinking water, aluminum based food additives, and aluminum cookware may have played a role.

Regarding other hair elements examined here, increased hair iron was a significant contributor to three of the teacher rated scales (acting-out, distractibility, and immaturity) and three of the parent rated scales (acting-out, distractibility, and withdrawal). While increased hair iron concentrations have been linked to behavioral disorders in children (Marlowe, et al., 1991), this assessment is compromised by the lack of basic information on the variability of iron levels in the hair and its correspondence with variability in the rest of the body's organs and tissues (Haddy, Czajka-Narins, Sky-Peck, and White, 1991). Increased hair concentrations of molybdenum and vanadium were also significant contributors to the teacher rated total scale score, while an increase in chromium was a significant contributor to parent rated total scale score. Chromium and vanadium hair concentrations were more than one

standard deviation below the theoretical normal ranges for both young children and adults, while hair-molybdenum values were more than one standard deviation below the young children norms. The clinical significance of the variance attributable to increased low hair concentrations of these elements in explaining the total scale scores is unknown. The relationship of these essential trace elements to the behavioral ratings may represent a statistical artifact or a nutritional peculiarity of the sample population.

The results of this study indicate that hair element concentrations are associated with nonadaptive behavior in young children. An issue suggested by these findings is whether intervention can ameliorate the condition. Schauss (1981b), Powers and Presley (1978), and Rimland and Larson (1981) imply that dietary changes and mineral supplements can normalize the trace element levels of most affected subjects and that such normalization may have a positive effect on disordered behavior.

Prior to summarizing the findings, it must be reiterated that hair element analysis is only a screening test and not a precise method of determining what is occurring in the body. Suspicions raised on hair analysis must always be confirmed by more precise and scientifically validated forms of testing before an exact diagnosis can be made.

We do not suggest that analysis of hair elements can be used to diagnose or predict behavior disorders. Rather the utility of the data suggests that possible mineral imbalances can exist among such subjects and should be further researched in terms of other biopsy materials, nutrient intake, and biochemical explanations as to the distributions of trace elements in various body compartments.

Twenty-one hair minerals were examined here. According to Passwater and Cranton's review of the literature (1983), hair minerals of proven clinical significance determined in this study are lead, cadmium, mercury, copper, zinc, calcium, magnesium, chromium, and nickel. Hair minerals suggested to have possible clinical significance are sodium and potassium, while the other hair minerals determined here have an unknown clinical significance because of an absence of scientific data. In summary, regression analysis indicated that the set of hair elements was significantly related to increased scores on the teacher and parent rated WPBIC scales, with lead being a major contributor to all of the dependent measures. We simply present this evidence to encourage others to examine element metabolism in young children in relationship to behavioral development.

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